STUDY PROTOCOL

1. Title: Immune-mediated necrotizing myopathy in the Portuguese Population - a multicentric nationwide study

2. Abstract

Immune-mediated necrotizing myopathy (IMNM) is a rare, distinct, and heterogeneous entity within the group of idiopathic inflammatory myopathies (IIM), that poses significant challenges in diagnosis and management. Understanding the interplay between clinical features, autoantibody expression, and outcomes is essential for tailoring personalized therapeutic strategies and improving prognosis. By leveraging data from the Portuguese rheumatic diseases registry (Reuma.pt), we aim to comprehensive characterize IMNM within the Portuguese population.

3. Background

Immune-mediated necrotizing myopathy (IMNM) is a rare, distinct, and heterogeneous entity within the group of idiopathic inflammatory myopathies (IIM), that poses significant challenges in diagnosis and management. The predominant clinical feature is acute or subacute and progressive proximal muscle weakness, associated with markedly elevated muscle enzymes levels. Extra-muscular involvement, particularly cardiac and pulmonary, has been described, albeit infrequently. Significant skin or lung involvement should suggest the possibility of another type of IIM.

Although IMNM was initially defined based on histopathological criteria, it emerged in 2003 as an individualized entity within IIM, with distinct phenotypes, myopathological characteristics, and association with specific autoantibodies (1). The most recent European Neuromuscular Centre (ENMC) criteria for IMNM divides this syndrome into three subtypes (table 1): anti- signal recognition particle (SRP) myopathy, anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) myopathy, and antibody-negative IMNM (2). The presence of elevated creatine kinase (CK) levels and proximal weakness is sufficient to diagnose the disease subtype in those patients that are positive for anti-SRP or anti-HMGCR autoantibodies; for those that are autoantibody-negative, it is necessary to obtain a muscle biopsy to demonstrate the characteristic features of a necrotizing myopathy.

Table 1. ENMC criteria for immune-mediated necrotizing myopathy.

<table>
<tr><th></th><th>Serologic criteria</th><th>Muscle Biopsy features</th><th>Clinical criteria</th></tr>
<tr><td>Anti-SRP myositis</td><td>Anti-SRP antibody</td><td rowspan="2">Not required</td><td rowspan="3">High creatine kinase
Proximal weakness</td></tr>
<tr><td>Anti-HMGCR myositis</td><td>Anti-HMGCR antibody</td></tr>
<tr><td>Antibody-negative IMNM</td><td>No myositis-specific antibody</td><td>Necrotic fibers
Different stages of:
- necrosis
- myophagocytosis
- regeneration
Paucilymphocytic infiltrate</td></tr>
</table>

Note: Drug / toxin-induced myopathy should be excluded.

These subtypes, most likely due to the role of the antibodies in the pathophysiology of the disease, present different clinical phenotypes, treatment responses, association with malignancy, and prognosis (3). Patients with anti-SRP antibodies generally present with more severe muscle weakness, with slow and sometimes incomplete recovery after treatment, and cardiac involvement is the most concerning extra-muscular manifestation, although rare (4, 5, 6). The risk of malignancy in this group seems comparable to the general population (4, 5, 7). Patients with anti-HMGCR antibodies frequently have a history of statin use, nonetheless statin-naïve patients appear to have a more refractory disease (8). Also, an increased risk of malignancy has been described on anti-HMGCR positive patients (8, 9). Seronegative patients are those with the highest risk of malignancy, which appears comparable to patients with dermatomyositis (6).

Histological, electromyographic, and magnetic resonance imaging (MRI) findings may be useful to characterize these patients.

As previously mentioned, according to the ENMC, a muscle biopsy is necessary to diagnose antibody-negative IMNM but is not strictly required for diagnosing anti-HMGCR or anti-SRP myopathy. However, this remains a topic of ongoing discussion. The 2017 European League Against Rheumatism/American College of Rheumatology Classification Criteria suggest that for adult patients without characteristic skin rashes, clinical and muscle biopsy features should be used to subclassify patients into Polymyositis/IMNM or Inclusion Body Myositis (IBM) (10). Furthermore, the histologic diagnosis of necrotizing myopathy - characterized by prominent myofiber necrosis in the absence of perifascicular atrophy or primary inflammation - can arise in different contexts, including in patients with anti-Mi2–positive dermatomyositis (DM) or antisynthetase syndrome (AS) (11, 12). Although relatively rare, these instances pose a challenge for classification. This highlights the ongoing uncertainty in classifying certain myopathies and underscores the value of muscle biopsies in ruling out other diagnoses.

The electromyogram (EMG) may be useful early in the diagnostic workup to confirm the presence of a myopathic pattern and to rule out other causes of muscle weakness.

MRI can show the distribution and severity of both active disease and chronic muscle damage in patients with IMNM, but has limited value in diagnosis because it poorly discriminates between IMNM and other types of myositis (13). In general, there is evidence of generalized muscle oedema, muscle atrophy, fatty replacement of muscle (that begins very early after the onset of the disease), minimal fascial oedema, and relatively less involvement of the anterior compartment compared with other types of myositis (13).

Among the IIM group, IMNM has one of the most severe forms of muscle involvement, with a long disease duration and a high risk of recurrence, often without complete recovery of muscle strength (5, 6, 9). Thus, despite significant advances in understanding the complexity of IMNM, there are still many aspects not fully elucidated, given its rare and heterogeneous nature.

The incidence of IIMs worldwide is estimated to be between 1.16 and 19 cases per million people each year, with a prevalence of 2.4 to 33.8 per 100,000 individuals (14). Because IMNM is a relatively new recognized IIM subtype and often mistaken for polymyositis, its exact epidemiology is hard to define and not fully known. Among IIM patients, 5-15% have anti-SRP antibodies, and 6-10% have anti-HMGCR antibodies (15). Nevertheless, estimates can most likely vary widely depending on the population studied and diagnostic criteria used.

Despite significant advances in understanding IMNM, several critical gaps remain. Most of the existing data derive from international studies, often with limited representation of specific

populations, including the Portuguese population. Furthermore, the rarity and heterogeneity of IMNM continue to pose challenges in its classification, particularly in distinguishing antibody-negative cases from other idiopathic inflammatory myopathies. Current knowledge about the interplay between immunological profiles, disease severity, and malignancy risk is also incomplete, leaving uncertainties that may hinder tailored management approaches. This study seeks to address these gaps by offering a comprehensive characterization of IMNM within the Portuguese population, contributing valuable data to the global understanding of this rare and complex disease.

4. Objectives

Primary objective:

To describe the proportion of patients that can be classified into one of the three different IMNM subgroups defined on the 224th ENMC International Workshop: Clinico-sero-pathological classification of immune-mediated necrotizing myopathies Zandvoort, The Netherlands, 14–16 October 2016.

Secondary objectives:

1. To determine the prevalence of IMNM in the Portuguese Inflammatory Myopathies Cohort (patients registered in Reuma.pt/Myositis protocol);
2. To thoroughly characterize the Portuguese IMNM cohort, regarding the following features:
    a. Clinical characteristics;
    b. Immunological profile (seronegative, anti-SRP positive, anti- HMGCR positive);
    c. Muscle biopsy, EMG and MRI features;
    d. Malignancy.
3. To associate immunological profiles with specific clinical features, namely severity of muscle weakness (CK and MMT8) and extra-muscular involvement;
4. To compare malignancy rates between immunological profiles;
5. To describe the proportion of patients that can be classified as having “possible”, “probable” and “definite” IIM according to the 2017 EULAR/ACR IIM classification criteria.

5. Methods

Type of study

Observational cross-sectional study

Case definition

Inclusion criteria:

- Patients with a clinical diagnosis of IMNM by their assisting rheumatologist;
- Patients registered in Reuma.pt/Myositis/IMNM protocol, with at least one registered clinical evaluation.

Exclusion criteria:

- Patients in whom another inflammatory Rheumatic and Musculoskeletal Disease (RMD) dominates the clinical picture (e.g., overlap syndromes and MSA-positive patients [other

than anti-SRP and anti-HMGCR] with evidence of necrotizing myopathy on muscle biopsy but with typical disease manifestations of other IIM subgroup, like dermatomyositis);
- Age less than 18 years.

Data collection

Portuguese Rheumatology centers will be invited to actively insert patients, fulfilling inclusion criteria, in Reuma.pt/Myositis/IMNM, and complete every missing variable required for this study. Data extraction will be conducted on the 1st of march of 2025. Data collection will start with exportation and subsequent exploratory analysis of the current data in Reuma.pt/Myositis. An anonymized Microsoft Excel document will be created and sent to every participating center, highlighting the Reuma.pt missing information. In the first phase of data completion, the center's designated local investigators will fill in the missing information. After the first phase of data completion is concluded, exportation and exploratory analysis of the data will be performed again. A new Microsoft Excel document will be created based on the exportation document.

Variables to be collected

- Number of patients registered in Reuma.pt/Myositis protocol

- Age (continuous variable)
- Sex (dichotomic variable: female 0; male 1)
- Date of loss of follow-up (date)
- Cause of loss of follow-up (categorical variable)
- Date of disease onset (date)
- First disease manifestation (free text; date)
- Date of diagnosis (date)
- Death (date)
    - Cause of death (free text)
    - Death related to IMNM (dichotomous variable: no 0; yes 1)
- Classification criteria: European NeuroMuscular Centre (ENMC), 2011 (each item, dichotomous variable: no 0; yes 1)

- Disease manifestations:
    - Myalgia/ myositis (dichotomic variable: no=0; yes=1)
    - Gottron's papules (dichotomic variable: no=0; yes=1)
    - Heliotrope rash (dichotomic variable: no=0; yes=1)
    - Raynaud's phenomenon (dichotomic variable: no=0; yes=1)
    - Digital ulcers (dichotomic variable: no=0; yes=1)
    - Oedema (dichotomic variable: no=0; yes=1)
    - Calcinosis (dichotomic variable: no=0; yes=1)
    - Periungual changes (dichotomic variable: no=0; yes=1)
    - Lipoatrophy (dichotomic variable: no=0; yes=1)
    - Arthralgia/ arthritis (dichotomic variable: no=0; yes=1)
    - Oesophageal involvement (dichotomic variable: no=0; yes=1)
    - Gastric involvement (dichotomic variable: no=0; yes=1)
    - Intestinal involvement (dichotomic variable: no=0; yes=1)
    - Heart involvement (dichotomic variable: no=0; yes=1)
    - Lung involvement (dichotomic variable: no=0; yes=1)
    - Others (free text)

- Immunological profile:
    - Anti-nuclear antibodies (ANA's) (dichotomic variable: negative=0; positive =1)
    - Anti-SRP (dichotomic variable: negative=0; positive=1)
    - Anti-HMGCR (dichotomic variable: negative=0; positive=1)
    - Another positive autoantibody (categorical variable)
    - Seronegative to all myositis-associated antibodies (MAA's) and myositis-specific antibodies (MSA's) (dichotomic variable: no=0; yes=1)
    - ANA titter and pattern (free text in the "Others" section from the Immunological characterization page)

- Other exams:
    - Muscle biopsy with myositis evidence (dichotomic variable: no=0; yes=1)
    - Muscle biopsy full report (free text in the ''Imagiology" page from ''complementary tests'' section)
    - Elevated muscle enzymes:
        - Creatine kinase (CK) (dichotomic variable: no=0; yes=1)
        - Lactate Dehydrogenase (LDH) (dichotomic variable: no=0; yes=1)
        - Aldolase (dichotomic variable: no=0; yes=1)
        - Aspartate Aminotransferase (AST) (dichotomic variable: no=0; yes=1)
        - Alanine Aminotransferase (ALT) (dichotomic variable: no=0; yes=1)
    - Muscle Enzymes level at baseline (to be introduced in ''Lab'' page of Complementary Tests section)
        - CK
        - LDH
        - Aldolase
        - AST
        - ALT
    - Myopathic alterations in EMG (dichotomic variable: no=0; yes=1)
    - MRI with myositis evidence (dichotomic variable: no=0; yes=1)

- Manual Muscle Testing (8 muscle groups) (MMT8) at baseline

  Note: When the Medical Research Council (MRC) 5-point scale is the sole option available in medical records, it should be converted to an expanded 10-point scale, following the strategy provided in the table below (table 2), outlined in International Myositis Assessment & Clinical Studies Group Disease Activity Core Set Measures (IMACS-CSM) Form 04: Manual Muscle Testing Procedures, available at https://www.niehs.nih.gov/research/resources/imacs/diseaseactivity.

Table 2. Manual muscle testing procedures - Key to Muscle Grading

	Function of the muscle	**Grade**		
No Movement	No contractions felt in the muscle	0	0	Zero
	Tendon becomes prominent or feeble contraction felt in the muscle, but no visible movement of the part	T	1	Trace
Test Movement	**Movement in horizontal plane**			
	Moves through partial range of motion	1	2-	Poor-
	Moves through complete range of motion	2	2	Poor
	Antigravity position			

	Moves through partial range of motion	3	2+	
Test Position	Gradual release from test position	4	3-	Fair-
	Holds test position (no added pressure)	5	3	Fair
	Holds test position against slight pressure	6	3+	Fair+
	Holds test position against slight to moderate pressure	7	4-	Good-
	Holds test position against moderate pressure	8	4	Good
	Holds test position against moderate to strong pressure	9	4+	Good+
	Holds test position against strong pressure	10	5	Normal

- Muscular involvement characterization
    - Proximal muscle weakness (dichotomic variable: no=0; yes=1)
    - Other muscle weakness (dichotomic variable: no=0; yes=1)
- Skin involvement characterization
    - Heliotrope rash (dichotomic variable: no=0; yes=1)
    - Gottron's sign or papules (dichotomic variable: no=0; yes=1)
    - Periungual capillary changes (dichotomic variable: no=0; yes=1)
    - Lipodystrophy (dichotomic variable: no=0; yes=1)
    - Calcinosis (dichotomic variable: no=0; yes=1)
    - Digital ulcers (dichotomic variable: no=0; yes=1)
    - Generalized subcutaneous oedema (dichotomic variable: no=0; yes=1)
    - Periorbital subcutaneous oedema (dichotomic variable: no=0; yes=1)
    - Malar/ facial rash (dichotomic variable: no=0; yes=1)
    - Shawl sign (dichotomic variable: no=0; yes=1)
    - Mechanic's hands (dichotomic variable: no=0; yes=1)
    - Alopecia (dichotomic variable: no=0; yes=1)
    - Vasculopathy lesions (dichotomic variable: no=0; yes=1)
    - Photo-sensitivity (dichotomic variable: no=0; yes=1)
    - Livedo reticularis (dichotomic variable: no=0; yes=1)
    - Panniculitis (dichotomic variable: no=0; yes=1)
    - Other skin involvement (dichotomic variable: no=0; yes=1)

- Organ involvement characterisation
    - Musculoskeletal involvement (dichotomic variable: no=0; yes=1)
        - Arthritis (dichotomic variable: no=0; yes=1)
        - Contractures (dichotomic variable: no=0; yes=1)
    - Gastrointestinal involvement (dichotomic variable: no=0; yes=1)
        - Dysphagia (dichotomic variable: no=0; yes=1)
        - Dysphonia (dichotomic variable: no=0; yes=1)
        - Abdominal pain or gastrointestinal ulcers (dichotomic variable: no=0; yes=1)
    - Lung involvement – interstitial lung disease (dichotomic variable: no=0; yes=1)
    - Heart involvement (dichotomic variable: no=0; yes=1)
    - Constitutional involvement characterization
        - Fever, as temperature > 38ºC (dichotomic variable: no=0; yes=1)
        - Weight loss (dichotomic variable: no=0; yes=1)
        - Fatigue (dichotomic variable: no=0; yes=1)
        - Raynaud's phenomenon (dichotomic variable: no=0; yes=1)
    - Neoplasia (dichotomic variable: no=0; yes=1)

        - Specific neoplastic diagnosis (categorical variable)
        - Date of onset (date)
        - Outcome (categorical variable: cure, remission, in treatment, persistent, death, unknown)
        - Date of outcome (date)

- Initial Patient Global Assessment (PtGA) (continuous variable)
- Initial Physician Global Activity (PGA) (continuous variable)
- Initial Health Assessment Questionnaire (HAQ) (continuous variable)
- Initial Extramuscular Activity assessed by the physician on a Visual Analog Scale (VAS) (to be introduced in ''Observation/Plan'' page of Today's Appointment section) (continuous variable)

- Previous and Ongoing Medications
    - Intravenous glucocorticoid pulses (dichotomous variable: no=0; yes=1)
    - Oral glucocorticoids (dichotomous variable: no=0; yes=1)
    - Intravenous immunoglobulin (dichotomous variable: no=0; yes=1)
    - Methotrexate (dichotomous variable: no=0; yes=1)
    - Azathioprine (dichotomous variable: no=0; yes=1)
    - Hydroxychloroquine (dichotomous variable: no=0; yes=1)
    - Rituximab (dichotomous variable: no=0; yes=1)
    - Mycophenolate mofetil / Mycophenolic acid (dichotomous variable: no=0; yes=1)
    - Cyclosporine (dichotomous variable: no=0; yes=1)
    - Tacrolimus (dichotomous variable: no=0; yes=1)
    - Oral cyclophosphamide (dichotomous variable: no=0; yes=1)
    - Intravenous cyclophosphamide (dichotomous variable: no=0; yes=1)
    - Others (dichotomous variable: no=0; yes=1)

- Other medications
    - Statin use (dichotomous variable: no=0; yes=1)
    - Type of Statin (categorical variable)

- Comorbidities of interest
    - Arterial hypertension (dichotomous variable: no=0; yes=1)
    - Dyslipidaemia (dichotomous variable: no=0; yes=1)
    - Obesity (BMI > 30 kg/m^2) (dichotomous variable: no=0; yes=1)
    - Diabetes Mellitus (dichotomous variable: no=0; yes=1)

- Tobacco and Alcohol habits
    - Tobacco (categorical variable: unknown, never smoked, former smoker, current smoker)
        - If former smoker, year in which stopped smoking (continuous variable)
        - Packs per day (continuous variable)
        - Years of smoking (continuous variable)
        - Packs per year (continuous variable)
    - Alcohol (categorical variable: unknown, never/social drinker, former drinker, current drinker)

<u>Variables to be created (transformed)</u>

- ENMC IMNM revised diagnostic criteria (2016) fulfilment (dichotomic variable: no=0; yes=1)
    - =1 if ''Elevated muscle enzymes: CK''=1 and ''Proximal muscle weakness''=1 and [''Anti-SRP''=1 or Anti-HMGCR=1 or description of necrotic fibers, paucilymphocytic infiltrate and different stages of necrosis, myophagocytosis, regeneration on the muscle biopsy report]
- ENMC IMNM revised diagnostic criteria (2016) anti-SRP subgroup (dichotomic variable: no=0; yes=1)
    - =1 if ''ENMC IMNM revised diagnostic criteria (2016)''=1 and ''Anti-SRP''=1
- ENMC IMNM revised diagnostic criteria (2016) anti-HMGCR subgroup (dichotomic variable: no=0; yes=1)
    - =1 if ''ENMC IMNM revised diagnostic criteria (2016)''=1 and ''Anti-HMGCR''=1
- ENMC IMNM revised diagnostic criteria (2016) antibody-negative subgroup (dichotomic variable: no=0; yes=1)
    - =1 if ''ENMC IMNM revised diagnostic criteria (2016)''=1 and [''anti-SRP''=0 and ''anti-HMGCR''=0] and description of necrotic fibers, paucilymphocytic infiltrate and different stages of necrosis, myophagocytosis, regeneration on the muscle biopsy report
- Possible IIM on 2017 EULAR/ACR IIM classification criteria (dichotomic variable: no=0; yes=1)
    - =1 if probability of IIM ≥50% and <55% (calculated using the online web calculator (www.imm.ki.se/biostatistics/calculators/iim)
- Probable IIM on 2017 EULAR/ACR IIM classification criteria (dichotomic variable: no=0; yes=1)
    - =1 if probability of IIM ≥55% and <90% (calculated using the online web calculator (www.imm.ki.se/biostatistics/calculators/iim)
- Definite IIM on 2017 EULAR/ACR IIM classification criteria (dichotomic variable: no=0; yes=1)
    - =1 if probability of IIM ≥90% (calculated using the online web calculator (www.imm.ki.se/biostatistics/calculators/iim)
- Death (dichotomic variable: no=0; yes=1)
    - =0 if "cause of loss of follow-up" and "date of loss of follow-up" are missing
    - =1 if "cause of loss of follow-up" = death
    - =9 if "cause of loss of follow-up" ≠ death
- Muscle involvement (dichotomic variable: no=0; yes=1)
    - =1 if "Myalgia/ myositis"
    - =1 in "Disease manifestations" or if "Muscle biopsy with myositis evidence" or "MRI with myositis evidence" =1 in "Other exams"
    - =1 or if "Proximal muscle weakness" in "Muscular involvement characterisation"
    - =1 or if "Dysphagia" or "Dysphonia" in "Gastrointestinal involvement" =1
    - MMT8, muscle enzymes elevation and EMG will not be considered to score "Muscle involvement" =1 because of their assumed lack of specificity
- Articular involvement (dichotomic variable: no=0; yes=1)
    - =1 if "Arthralgia/ arthritis" =1 in "Disease manifestations" or if "Arthritis" in "Musculoskeletal involvement characterisation" =1 or if "Worst swollen joint count" >0
- Skin involvement (dichotomic variable: no=0; yes=1)
    - =1 if "Gottron's papules", "Heliotrope rash", "Calcinosis", "Oedema", "Periungual changes" or "Lipoatrophy" =1 in "Disease manifestations" or if "Worse DAS skin" > 0 or if at least one variable =1 in "skin disease characterisation"

- Gastrointestinal involvement (dichotomic variable: no=0; yes=1)
    - =1 if “Oesophageal involvement” =1 in “Disease manifestations” or if “Gastric involvement” =1 in “Disease manifestations” or if “Intestinal involvement” =1 in “Disease manifestations” or if “Gastrointestinal involvement” in “Organ involvement characterisation” =1
- Lung involvement (dichotomic variable: no=0; yes=1)
    - =1 if “Lung involvement” =1 in “Disease manifestations” or if “Lung involvement – interstitial lung disease” in “Organ involvement characterisation” =1
- Heart involvement (dichotomic variable: no=0; yes=1)
    - =1 if “Heart involvement” =1 in “Disease manifestations” or if “Heart involvement” in “Organ involvement characterisation” =1
- Gottron’s papules or sign (dichotomic variable: no=0; yes=1)
    - =1 if “Gottron’s papules” =1 in “Disease manifestations” or if “Gottron’s sign or papules” in “skin disease characterisation” =1
- Heliotrope rash (dichotomic variable: no=0; yes=1)
    - =1 if “Heliotrope rash” =1 in “Disease manifestations” or if “Heliotrope rash” in “skin disease characterisation” =1
- Subcutaneous oedema (dichotomic variable: no=0; yes=1)
    - =1 if “Oedema” =1 in “Disease manifestations” or if “Generalized subcutaneous oedema” in “skin disease characterisation” =1 or if “Periorbital subcutaneous oedema” in “skin disease characterisation” =1
- Calcinosis (dichotomic variable: no=0; yes=1)
    - =1 if “Calcinosis” =1 in “Disease manifestations” or if “Calcinosis” in “skin disease characterisation” =1
- Periungual changes (dichotomic variable: no=0; yes=1)
    - =1 if “Periungual changes” =1 in “Disease manifestations” or if “Periungual capillary changes” in “skin disease characterisation” =1
- Digital ulcers (dichotomic variable: no=0; yes=1)
    - =1 if “Digital ulcers” =1 in “Disease manifestations” or if “Digital ulcers” in “skin disease characterisation” =1
- Raynaud’s phenomenon (dichotomic variable: no=0; yes=1)
    - =1 if “Raynaud’s phenomenon” =1 in “Disease manifestations” or if “Raynaud’s phenomenon” in “constitutional involvement characterisation” =1

<u>Variables to be created (calculated)</u>

- Age at disease onset (continuous variable)
    - (“Date of first symptom” – “Date of birth”) in days / 365
- Age at diagnosis (continuous variable)
    - (“Date of diagnosis” – “Date of birth”) in days / 365
- Diagnostic delay in years (continuous variable)
    - (“Date of diagnosis” – “Date of first symptom”) in days / 365
- Disease duration in years at the time of death (continuous variable)
    - (“Date of loss of follow-up” – “date of first symptom”) in days / 365, if “cause of loss of follow-up” = death
- Disease duration in years up to the last follow-up (continuous variable)
    - (“Date of the last appointment” – “date of first symptom”) in days / 365, if “cause of loss of follow-up” ≠ death

<u>Statistical analysis</u>

The data will be analyzed using Statistical Package for the Social Sciences (SPSS) version 28.0.1.0 (SPSS, Inc., Chicago, IL, USA).

Proportion of patients with IMNM that can be classified into each of the three IMNM subgroups defined on the 224th ENMC International Workshop will be presented using absolute number and percentage.

Prevalence of IMNM will be calculated dividing the number of cases (defined elsewhere) by the total of patients included in Reuma.pt/Myositis protocol.

Descriptive statistics will be presented as mean ± standard deviation for continuous and normal variables, as median (interquartile range) for continuous non-normal variables, and as absolute and relative frequencies for categorical variables. A descriptive analysis of IMNM patients will be performed regarding the following features:

a) Sex, age at disease onset and diagnosis, disease duration;
b) Organ involvement, including muscular, skin, gastrointestinal, lung, heart, musculoskeletal and constitutional involvement;
c) Immunological profile;
d) Muscles enzymes and MMT8 at baseline;
e) Muscle biopsy / EMG / MRI changes;
f) Statin, tobacco and alcohol exposure;
g) Comorbidities, including arterial hypertension, dyslipidaemia, obesity and diabetes mellitus
h) Malignancy;
i) Treatment exposure;
j) Death.

IMNM subgroups (Seronegative, anti-SRP positive, anti- HMGCR positive) will be compared regarding:

a) age at disease onset, age at diagnosis, muscle enzymes level and MMT8 using Student's t test/Mann-Whitney Test or ANOVA or Kruskal-Wallis, as appropriate;
b) sex, statin/tobacco/alcohol exposures, comorbidities, muscle biopsy/EMG/MRI changes, organ involvement, malignancy and death using ChiSquare Test/Fischer's Exact Test

Statistical significance will be set at $p < 0.05$.

Proportion of patients that can be classified as having "possible", "probable" and "definite" IIM according to the 2017 EULAR/ACR IIM classification criteria will be presented using absolute number and percentage.

6.1. Expected results

With this study we expect to thoroughly characterize the national cohort of patients with IMNM within the Portuguese population. We expect to create awareness of the available data and hope to foster further clinical research work based on this cohort, which can ultimately lead to improved diagnosis, treatment, and management strategies for affected individuals. In terms of clinical subtypes and autoantibodies prevalence, we expect our cohort to be similar to international cohorts of similar latitudes.

6.2. Possible limitations

Sample size

IMNM is a very rare group of diseases, and therefore data sets tend to be small. There will be an effort to include the largest number of rheumatology centers, regardless of size, nature or geographic location, in order to maximize our sample size and have a cohort that is representative of the general Portuguese IMNM population. Besides, we will consider using measures of effect size to better interpret statistically non-significant results.

Internal and external validity

Since this study focuses on a specific cohort (Reuma.pt/Myositis/IMNM) and it will potentially include the whole target population, we do not expect major issues with external validity. Only patients registered in Reuma.pt/Myositis who do not have a clinical characterization will be excluded. Therefore, we expect that most patients within our target population will be included in this study. Moreover, we will ask all participating centers to complete the missing information to avoid the exclusion of any patient. However, our results may not be generalizable to other cohorts with different genetic backgrounds. In order to get maximum representativity, we expect not only tertiary but also secondary centers to participate in this project. Centers that actively collaborate in the project may designate project co-authors, irrespective of the number of eligible patients.

Missing data / Typing errors

All participating centers will be asked to complete the missing information with data from patients' medical records whenever such information is available. To minimize the influence of typing errors, we will make an exploratory evaluation of the data extracted from Reuma.pt. Outlier cases will be individually analyzed.

Publication bias

We expect to publish the results of our study, irrespective of the significance of our results.

7. Calendar of tasks

- Literature review, study design and elaboration of research protocol: April – August 2024
- Submission of research protocol to Reuma.pt and Ethics Commission: September 2024
- Invite all national centers to participate in the project: January 2025
- Data extraction and first Microsoft Excel document compilation: 1st march 2025
- Data completion by all participating centres: March 2025
- Data extraction and second Microsoft Excel document compilation: May 2025
- Data analysis: May-June 2025
- Final report and abstract submissions for presentation at national/international congresses as well as publication: June-October 2025

8. Ethical considerations

The study will be conducted according to the principles of the Declaration of Helsinki (revised in Fortaleza – 2013) and will be submitted for evaluation and approval to the Ethics Committee of Unidade Local de Saúde do Alto Minho (ULSAM) and the Reuma.pt National Committee.

This work's databases and all steps of the research process will be fully anonymized.

All patients must have signed the Reuma.pt informed consent to be included.

9.1. Proponents

Anita Cunha[1], Maria Pontes Ferreira[1]

9.2. Research team

Anita Cunha[1], Maria Pontes Ferreira[1], Francisca Guimarães[1], Catarina Dantas Soares[1], Susana Almeida[1], Eduardo Dourado[2], Filipa Teixeira[1], José Tavares-Costa[1], Daniela Peixoto[1]

9.3. Institutions

The project will be coordinated by the proponent (first author) and the senior author (last author), with the following affiliations:

1. Rheumatology Department, Unidade Local de Saúde do Alto Minho (ULSAM), Ponte de Lima, Portugal.

2. Rheumatology Department, Unidade Local de Saúde da Região de Aveiro, Aveiro, Portugal.

The project is open to all national centres willing to participate, which will all be formally invited.

9.4 Co-authorship

Clinicians who actively collaborate in the project will be co-authors, according to the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (Vancouver Convention). Authors will be listed in the Title Page of the manuscript, according to the definitions below:

- The Title Page will include First Author (lead author of the project), last author (senior author of the project) and researchers from each centre, listed in descending order according to the number of valid patients finally included per centre and the rules defined below.

- Always considering the limitations in the total number of co-authors in the title page accepted by the corresponding Journal, a minimum of 1 valid patient will be the criterion to be included on the title page of the manuscript, with a maximum of 2 co-authors per participating centre. Subsequently, from 20 patients, an additional co-author can be included in the collaborative authorship list or title page.

In addition to patient inclusion, other factors may be considered for defining authorship/order of authorship, including: contribution to data collection, project conception, input in study design, data analysis, and drafting and critical revision of the final manuscript.

10.1. Budget

Reuma.pt data exportation (to be defined).

10.2. Conflicts of interest

There are no conflicts of interest to be declared.

11. References

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